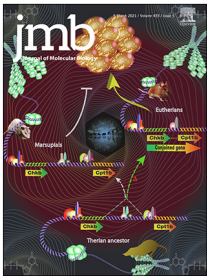
Review Article



Collaboration through chromatin: motors of transcription and chromatin structure

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Abstract

Packaging of the eukaryotic genome into chromatin places fundamental physical constraints on transcription. Clarifying how transcription operates within these constraints is essential to understand how eukaryotic gene expression programs are established and maintained. Here we review what is known about the mechanisms of transcription on chromatin templates. Current models indicate that transcription through chromatin is accomplished by the combination of an inherent nucleosome disrupting activity of RNA polymerase and the action of ATP-dependent chromatin remodeling motors. Collaboration between these two types of molecular motors is proposed to occur at all stages of transcription through diverse mechanisms. Further investigation of how these two motors combine their basic activities is essential to clarify the interdependent relationship between genome structure and transcription.

Introduction

Elucidating the mechanisms of eukaryotic transcription is a major pursuit with deep implications for understanding the regulation of cellular states, development, and disease. Transcription in eukaryotes occurs in the context of chromatin: the crowded and highly regulated structures that package DNA. The fundamental unit of chromatin is the nucleosome: a highly stable structure, which wraps 145–147 bp of DNA around an octamer of histone proteins.[1,2] Because of the tight DNA wrapping and high stability of nucleosomes, chromatin presents a major barrier to transcription by RNA polymerases (Pols).[3] Indeed, transcription by bacterial RNA polymerase and eukaryotic RNA Pol II is greatly inhibited on nucleosomal templates in vitro at physiological salt concentrations.[4,5] Furthermore, several factors which directly reshape chromatin structure, including ATP-dependent chromatin remodeling enzymes, also regulate transcription in vitro and in vivo.[6,7] These findings have shaped a view of nucleosomes as generic repressors of transcription, with their regulated disruption enabling transcription.

However, several findings challenge this simple view of the role of nucleosomes and more generally, chromatin structure in transcription. In contrast to Pol II, bacteriophage SP6 and eukaryotic Pol III are only modestly inhibited on nucleosome-containing templates at physiological salt.[8,9] Furthermore, transcription by these polymerases does not necessarily involve nucleosome eviction.[8,9] These observations suggest that transcription need not be incompatible with the presence of nucleosomes. Additionally, at slightly higher salt concentrations, Pol II efficiently transcribes through nucleosomes with only the loss of

a single H2A/H2B dimer.[4] However, some sub-nucleosome particles, which physically occlude less DNA, inhibit transcription when compared to a complete nucleosome.[10] Finally, it has been shown that transcriptionally silenced regions can be accessible to digestion by nucleases and further that nucleosome plasticity promotes formation of transcriptionally repressed heterochromatin.[11,12] Together these results shape a more nuanced view of chromatin in transcription as a dynamic platform that has coevolved with RNA polymerase and other nuclear proteins to enable complex, tightly regulated gene expression programs.

Key to understanding how transcription operates on chromatin is a thorough understanding of how chromatin structure can be reorganized. The SWI2/SNF2 superfamily of ATP-dependent chromatin remodeling enzymes play an essential role in facilitating DNA-based processes (including transcription) by directly reshaping chromatin at the level of individual nucleosomes.[6,7] Like eukaryotic RNA polymerase, remodelers are large, multisubunit molecular machines which carry out complex and highly regulated reactions. Compared to RNA polymerase, our understanding of the basic mechanisms of remodelers is less mature. Even less understood is how remodeler activity is coordinated with RNA polymerases and other factors to both faithfully regulate transcription while maintaining genome architecture. However, recent structures of both remodelers and RNA polymerase with nucleosomes, together with prior biochemical data enable models for how these motors might collaborate.

In this review we first briefly summarize our current understanding of the core process of transcription and its regulation by elongation factors. We then discuss how this basic process is influenced by the presence of chromatin. Specifically, we focus on how the molecular motor at the heart of transcription, RNA polymerase, may accomplish its activity despite the physical constraints of the nucleosome. This is both due to an inherent chromatin remodeling capability of RNA polymerase and due to its collaboration with other factors including remodelers. We further compare our understanding of the chromatin remodeling activity of RNA polymerase to that of ATP-dependent chromatin remodelers and highlight open questions. Finally, we review how these ATP-dependent chromatin remodelers and RNA polymerases might collaborate.

The Nucleosome: beyond a barrier

Although transcription by all three eukaryotic RNA polymerases on chromatin templates is generally inhibited, the nature and strength of this barrier varies. This depends not only on properties of the polymerase and its associated factors but also the structural properties of the nucleosome itself. Since the detailed structure of the canonical nucleosome was solved, a wealth of biophysical studies shed some light on the core physical properties of the nucleosome, which provides insights into how molecular motors disrupt its structure (Figure 1).[13]

The canonical nucleosome is an ~0.2 MDa complex and contains ~147 bp of DNA bound to an octamer of histone proteins: 2 copies each of histones H2A, H2B, H3, and H4 (Figure 1(B)). Each histone possesses both a structured domain, which folds cooperatively with the other histones to form a globular core, and unstructured tails.[2] Histones assemble in a stepwise fashion on DNA with two heterodimers of H3/H4 first depositing to form a core tetramer followed by two heterodimers of H2A/H2B. DNA wraps around the symmetric globular core ~1.7 times with the unstructured tails projecting out of the core.[1] This wrapping is stabilized by electrostatic contacts between basic residues in the histone core and the DNA phosphodiester backbone. With an average pI of ~11, histone proteins have a high net negative charge at physiological pH that facilitates their interaction with DNA.[14] However, a key acidic surface on H2A/H2B, known as the acidic patch, plays an important role as a recognition surface for many chromatin proteins (Figure 1(A)).[15] Additionally, wrapping of DNA around the nucleosome introduces a single negative supercoil constrained over the length of the nucleosomal DNA.[16] This is because the >-1 writhe associated with wrapping is compensated by reduced DNA twist within the nucleosome.[17] Topological changes in nucleosomal DNA are important for the stability of nucleosomes as octamer assembly on positively supercoiled DNA templates is disfavored.[18] Correspondingly, nucleosomal DNA subjected to torsional stress from positive supercoiling reduces nucleosome stability.[19]

The nucleosome is thermodynamically a very stable structure. In vitro nucleosomes remain intact under physiological salt concentrations at low micromolar concentrations even when heated to temperatures as high as 65 °C.[20] At the same time, several dynamic transitions can be observed in nucleosomes without the addition of other factors (Figure 1(C)). DNA near the entry/exit (SHL ± 7) can transiently peel off the surface off the histone octamer exposing the underlying DNA.[21,22] The histone H2A/H2B dimer can also associate with this unpeeled DNA and undock from the histone core.[23] Electron cryo-microscopy (cryo-EM) structures suggest that such dynamic changes involve intermediates with subtle conformational changes in the histone octamer.[24] Reversible dissociation of the H2A/H2B dimer also occurs and can result in stable sub-nucleosome structures, which wrap less DNA than a full nucleosome.[25] Additionally, it is proposed that the unstructured histone tails may associate with nucleosomal DNA influencing its exposure

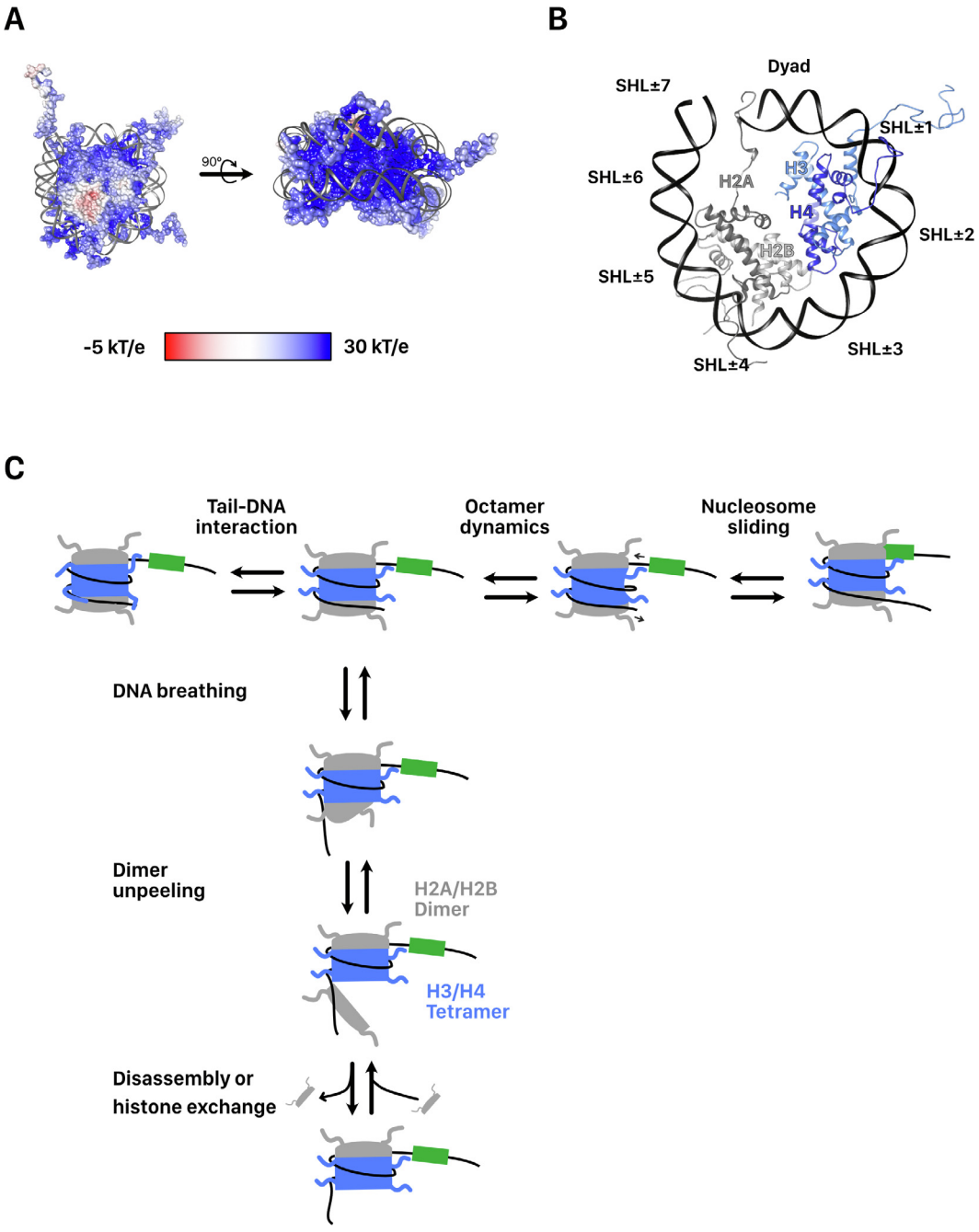


Figure 1. Core properties of the nucleosome. (A) Nucleosome core particle with the surface of the histone octamer colored according to the gradient specified below. The unit kT/e refers to the energy associated with placing a unit charge at a specific location. (B) Ribbon diagram of one pseudosymmetrical half of the nucleosome. Locations of nucleosomal DNA are specified according to their SuperHelical Location (SHL) with each location being 10 bp from the dyad axis of symmetry. (C) Dynamic states of a nucleosome in solution. Nucleosomes exist in equilibrium between many possible states. Transition between states may involve conformational changes in the histone octamer (e.g. during DNA breathing).

and dynamics.[26,27] Finally, nucleosomes have also been observed to spontaneously reposition along DNA in a process known as nucleosome sliding.[28] Molecular dynamics simulations suggest that subtle local changes in DNA twist could allow the resetting of histone-DNA contacts around the octamer by ~1 bp increments.[29] CryoEM studies combined with histone-histone crosslinking suggest that dynamic rearrangements in histone conformation also facilitate nucleosome sliding.[30]

The intrinsic properties of the nucleosome can be modulated by several factors. Sequences that can

accommodate the DNA deformations associated with nucleosome formation promote nucleosome stability. Such sequences are often associated with AT dinucleotides at sites where the minor groove of DNA faces the histone octamer and GC dinucleotides at sites where the major groove of DNA faces the histone octamer.[31] This is because AT and GC dinucleotides favor compression of the minor and major grooves respectively, which occurs upon interaction with the histone octamer.[32] In contrast, continuous poly dA:dT tracts seen at some promoters disfavor nucleosome stability.[33] Post-translational modifications on histones can also influence the structure, dynamics, and stability of the nucleosome.[34] Acetylation of histone tails has small effects on DNA unpeeling and histone tail accessibility, while phosphorylation and acetylation of the H3 core may have more substantial effects.[34,35] Several variant histones also exist and can be incorporated instead of canonical histones, which can alter nucleosome properties. For example, histone H2Az is an H2A variant that is is particularly enriched at the +1 nucleosome relative to the transcription start site (TSS).[36] H2Az-containing nucleosomes are less stable to mechanical stress, which may facilitate transcription elongation (discussed further below).[37]

Transcription through nucleosomes

RNA polymerase: A nucleotide dependent chromatin remodeler

In order to understand how RNA polymerase negotiates nucleosome structure, an understanding of the basic structure and mechanism of RNA polymerase is required. Transcription in Eukaryotes is carried out by one of three large (~0.5–1 MDa) multisubunit RNA Polymerases: Pol I, II, and III (Figure 2(A)).[38,39] Most protein coding genes are transcribed by RNA Pol II. In contrast Pol I and III mostly transcribe noncoding genes, with rRNA being transcribed by Pol I and 5S rRNA, tRNA and other small RNAs being transcribed by Pol III. All three polymerases contain a core of 10 polymerase-specific subunits that share structural homology to prokaryotic multisubunit RNA polymerases.[38] This structure resembles a “claw” with three pincers called the “clamp”, “lobe”, and “jaw” that surround the DNA (Figure 2(A)). The clamp and jaw specifically engage the downstream DNA and feed it into the catalytic center.[38] In addition to these core subunits, the three polymerases also possess a flexible heterodimeric “stalk module” that binds to the core clamp module. The stalk module can make interactions with transcriptional regulators and the nascent RNA. Though considered part of the core polymerase, under certain circumstances the stalk module may need to dissociate during transcription by Pol II with consequences on RNA processing.[40,41]

While many structural aspects are shared across all three eukaryotic RNA polymerases there are some key differences. The largest subunit of Pol II contains a repetitive extension at its C-terminus called the CTD, which is phosphorylated or dephosphorylated in specific patterns at different stages of transcription to help recruit transcriptional regulators.[42] Pol I and III utilize specific lobe-binding (LB) modules, which resemble the Pol II initiation factor TFIIF.[38] The LB module binds to the core subunits A12.2 in Pol I and C11 in Pol III. These core subunits contain domains that resemble the elongation factor TFIIS that is typically used by Pol II and stimulate elongation using a similar mechanism.[43] Pol III also uniquely contains a heterotrimeric module that assists in transcription initiation.[44]

To enable efficient elongation, RNA polymerase must function as a processive molecular motor, efficiently translocating along DNA. Several single-molecule studies have helped uncover the mechanical properties of RNA polymerases. Pol II can transcribe against forces of up to ~6.5 pN while Pol I can withstand somewhat higher forces (~9.5 pN).[45] Translocation is thought to function through a brownian ratchet mechanism, where the polymerase can freely diffuse one-dimensionally along the RNA·DNA hybrid forward or backward relative to the 3′ end of the nascent RNA (Figure 2(B)).[46,47] The irreversible incorporation of a complementary ribonucleotide provides the energy to bias forward translocation. Despite such a mechanism for directionality, pausing and backtracking of the polymerase is frequent and often represents a rate-limiting barrier to transcription.[46] Pause locations are influenced by properties of the DNA sequence being transcribed, the secondary structure of the nascent RNA, and by barriers such as nucleosomes.[46,48] While backtracking and pausing are often reversible, in some instances pausing can be irreversible and require the action of other factors to be overcome. As a result, elongation factors have evolved that physically associate with polymerase and prevent or resolve backtracked pauses. The elongation factor TFIIS helps resolve irreversibly backtracked/paused polymerase by catalyzing the endonucleolytic cleavage of the extruded 3′-end of the nascent RNA (Figure 2(B)). This resets the 3′ end of the nascent RNA at the pause location and allows further rounds of elongation.

The unwinding of DNA by RNA polymerases generates torsional stress and is a major source of DNA supercoiling throughout the genome.[49] Transcription generates negative torsional stress upstream, which is expected to stabilize nucleosomes, and positive stress downstream of the polymerase, which is expected to destabilize nucleosomes (Figure 2(B)). If unresolved, torsional stress can hinder both the continued elongation and the stability of the chromatin template.[50] As a result,

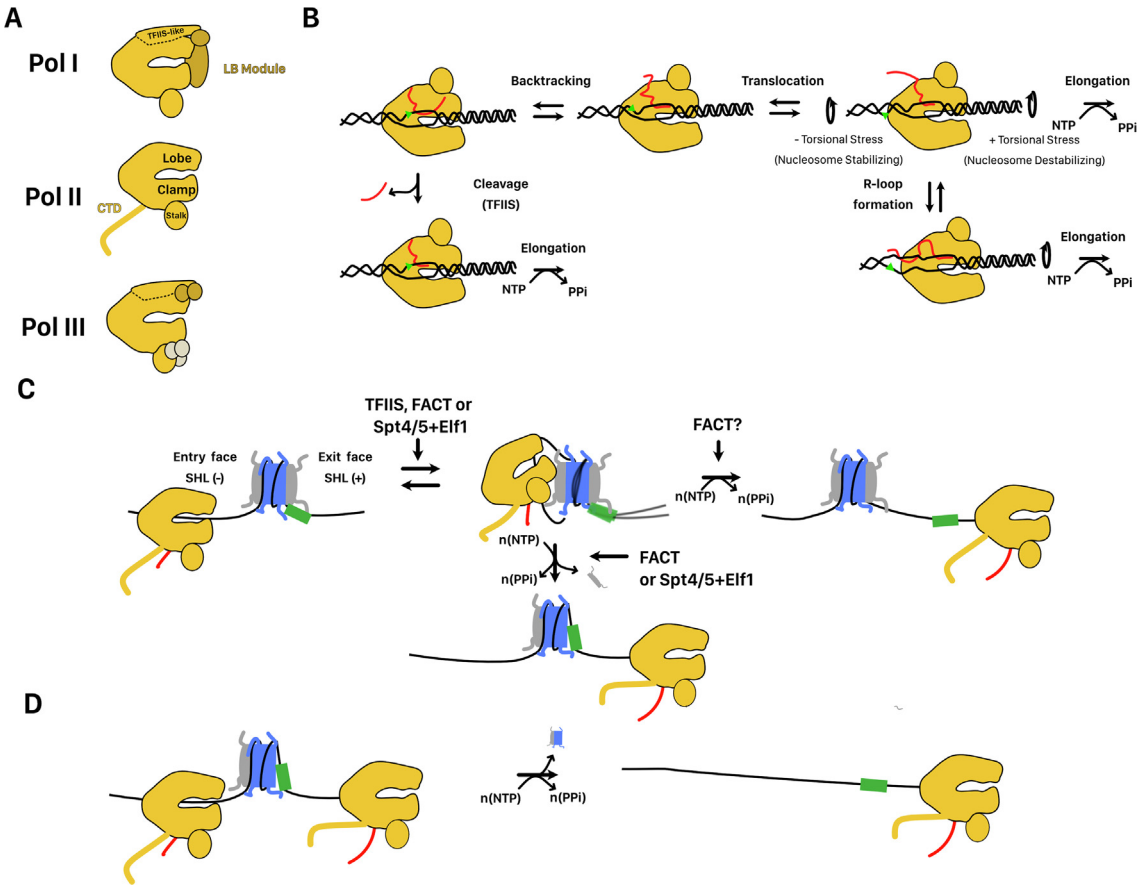


Figure 2. Mechanisms of nucleosome remodeling by RNA polymerases. (A) Cartoon representations of the three eukaryotic RNA polymerases. The lobe, clamp, and stalk domains that are shared between all three polymerases are labeled on Pol II. The TFIIS-like domain of the lobe shared by Pol I and III is labeled next to Pol I. This domain is bound by different Lobe-Binding (LB) modules. Finally, the unique CTD of Pol II is also shown. (B) Core mechanism of elongation shared by RNA polymerases. The green triangle is used as a reference point for polymerase translocation. Polymerase can either translocate 1 nt forward or pause and backtrack. DNA unwinding is associated with torsional stress ahead of and behind the transcription bubble. Stress behind the bubble can be absorbed by formation of a stable RNA-DNA hybrid called an R-Loop. Backtracked polymerase can be resolved by cleavage of the nascent transcript by TFIIS or the TFIIS-like domains of Pol I and III. Forward elongation is biased by irreversible incorporation of a complementary nucleotide. (C) Mechanism of polymerase passage through the nucleosome. The green box is used as a reference location on the DNA. As polymerase translocates into the nucleosome core, it unpeels DNA at the entry side until it reaches the dyad. Once at the dyad, further passage requires DNA unpeeling at the exit side and loosening of histone DNA contacts downstream of the dyad. Further passage is associated with either octamer transfer upstream of its current site or dissociation of an H2A/H2B dimer from the exit side. Elongation factors assist entrance into the nucleosome and may bias the outcome of Pol II passage. (D) Polymerase passage through a hexasome by Pol II is associated with complete disassembly of the hexasome.

relief of DNA supercoiling by topoisomerases plays an important role in continued transcription and maintenance of chromatin structure.[50,51] Additionally, stable RNA:DNA hybrids formed between the nascent RNA and the DNA upstream of the polymerase, known as R-loops, may also play a role in absorbing negative torsional stress (Figure 2 (B)).[49]

Eukaryotic RNA polymerases have been long appreciated as remodelers of nucleosome structure.[52] Early experiments of transcription on short nucleosome-containing templates suggested two mechanisms of remodeling linked to the type of polymerase transcribing. Transcription by Pol III is only modestly inhibited at physiological salt concentration and transfers intact histone octamers largely in cis upstream of its original position.[9] Like Pol III, nucleosomes also present a more modest barrier to transcription by Pol I under physiological conditions, although nucleosome transfer activity has not yet been reported.[53] In contrast, Pol II is almost completely inhibited at physiological salt concentrations.[4] At slightly higher salt concentrations, which loosens histone-DNAcontacts, Pol II is able to more

efficiently transcribe through the nucleosome with the dissociation of a single H2A/H2B dimer and without net change in nucleosome position.[4,54] This matches well with *in vivo* observations that H2A/H2B dimers are more readily exchanged at active genes than H3/H4.[55] Interestingly, E. coli RNA polymerase transcribes through nucleosomes with very similar properties as Pol II suggesting that nucleosome organization may have evolved to capitalize on an essential and ancient property of multisubunit RNA polymerases.[5]

More recent experiments suggest that the mechanisms of Pol II and III may fundamentally be more similar than different. On longer DNA templates, Pol II transfers nucleosomes in cis upstream of its original location.[56,57] Apparent nucleosome transfer intermediates with Pol II have been visualized using atomic force microscopy and CryoEM.[57,58] Also, with TFIIF and TFIIS, Pol II more efficiently transcribes through nucleosomes raising the possibility that the TFIIF-like LB module or the TFIIS-like domains in the lobes of Pol I and III are responsible for their higher efficiency.[59] Indeed, deletion of the LB modules or the TFIIS-like domain from Pol I reduces its ability to transcribe through a nucleosome.[60] In light of these observations, it might be more useful to think of the Pol III and Pol II mechanisms as two potential pathways of nucleosome disruption that might be taken by eukaryotic polymerases (Figure 2(C)).

Of the three polymerases, the mechanism of transcription through chromatin is most understood for Pol II. Analyses of the pause sites within the nucleosome provide some clues for how this polymerase remodels nucleosomes.[10,61] At low salt concentrations, Pol II is predominantly paused at the entry side of the nucleosome, suggesting that the tight wrapping of the nucleosome blocks transcription. At higher salt concentrations, Pol II pause sites progressively invade further into the nucleosome up until just before the dyad. Pol II paused prior to the nucleosome dyad exposes entry DNA to cleavage by restriction enzymes.[10] Together, these results suggest that entry of Pol II into the nucleosome is associated with unpeeling of nucleosomal DNA at the entry side. Optical trapping experiments combined with kinetic modeling have supported the notion that RNA polymerase takes advantage of spontaneous DNA breathing in order to enter and ratchet through the nucleosome.[35,56] Strangely, however, hexasomes lacking a single H2A/H2B dimer at the entry side, and thus unwrapped, are transcribed less-efficiently than full nucleosomes.[10] This suggests that the entry side dimer may be important in establishing intermediates required for passage through the nucleosome. In contrast, hexasomes lacking a dimer on the nucleosome exit side are transcribed more efficiently than nucleosomes and are completely disassembled by Pol II passage (Figure 2(D)).[10] Hexasomes, produced by Pol II passage are more efficiently transcribed than nucleosomes, suggesting that Pol II preferentially displaces the exit side dimer.[62] This is consistent with genome-wide observations that hexasomes within the gene body primarily lack an H2A/H2B dimer on the promoter-distal side of the nucleosome.[63]

Raising salt concentration or adding elongation factors shifts the location of Pol II pauses further into the nucleosome up until SHL −2/−1.[61] Beyond this location, few nucleosome-dependent pause sites are observed. This suggests that entering the nucleosome represents a greater barrier than exiting the nucleosomes. Interestingly, Pol II pausing in vivo is enriched right before the nucleosome dyad, further suggesting that transiting specifically through the dyad may be the dominant barrier to elongation.[61] Consistent with this idea, mutating a histone-DNA contact near the dyad both reduces pausing near the dyad and improves transcription efficiency.[35,64] What structural changes are necessary to allow Pol II to pass through the dyad? Since removal of the exit side dimer facilitates passage of Pol II, transiting beyond the dyad may also involve unpeeling of DNA from the exit side. Consistent with this possibility, increasing the length of DNA exiting the nucleosome, which promotes DNA breathing, allows Pol II to progress deeper into the nucleosome.[65] Pol II progression may allosterically loosen histone-DNA contacts ahead of the polymerase. Indeed, Pol II arrested near SHL −2 shows increased restriction site accessibility downstream of the polymerase near SHL +1.[10] Additionally, DNA sequences near SHL +2 appear to influence the ability of polymerase to transit. Interestingly, sequences that promote pol II passage do not necessarily destabilize nucleosomes. These observations suggest that Pol II passage may require the formation of a specific sequence-dependent intermediate to pass beyond the dyad.

Recent high resolution CryoEM snapshots of Pol II transcribing on nucleosomal templates provide new clarity to earlier biochemical observations.[58,66–68] As Pol II initially approaches the nucleosome, DNA at the entry site unpeels from the octamer consistent with biochemical and biophysical observations.[58,66] In this initial encounter the lobe and clamp regions of the core make additional contacts with nucleosomal DNA at the dyad.[66] Further progression peels more entry side DNA from the surface of the octamer. Once Pol II reaches SHL-1, the lobe region of Pol II makes contact with the H2A/H2B dimer, providing an explanation for the retention of the entry side dimer. Major pause sites internal to the nucleosome are immediately adjacent to histone-DNA contacts, suggesting that breaking these contacts is required for further progression. The precise relative motion of Pol II with respect to histones and/or DNA between nucleosomal pause sites is unclear and is an important area of study.[69–71] Some reconstructions of the SHL −1 pause also revealed the presence of an

additional DNA molecule replacing the region of DNA unpeeled from the octamer, which may represent a histone transfer intermediate. Consistent with this, close inspection of the raw Cryo-EM micrographs show evidence for nucleosomes invading this complex.[58]

Regulation of RNA polymerase-mediated nucleosome remodeling

Several features of nucleosome structure play an important role in controlling elongation through the nucleosome. As already mentioned, DNA sequence can influence RNA polymerase progression through the nucleosome.[61] Histone tails repress the ability of Pol II to transit through the nucleosome largely through their effect on restricting DNA breathing.[35,72] Several post translational modifications of the histone proteins play positive roles in Pol II passage. Acetylation of histone tails improves Pol II passage by modestly increasing DNA breathing.[35] Histone modifications may also directly affect core histone-DNA interactions. Poly ADP-ribosylation of histones, which is associated with elongation, appears to globally loosen histone-DNA contacts.[73] Importantly, not all transcription-associated histone modifications play a direct role in the progression of Pol II through the nucleosome, as has been suggested for H3K4 trimethylation.[74] Instead, these may regulate transcription by impacting higher order chromatin organization or the recruitment of additional regulators. The incorporation of certain histone variants can also facilitate passage through the nucleosome (as discussed later).

As mentioned before, elongation factors promote the progression of polymerases through the nucleosome. Pol II-nucleosome structures suggest that transcription intermediates can accommodate association of several elongation factors including NELF, Paf1, and TFIIS.[66] Transcription of nucleosome-containing templates with Pol II and elongation factors both shifts pause sites deeper into the nucleosome and improves overall transit efficiency. CryoEM structures of Pol II-nucleosome intermediates with the elongation factors Spt4/5 and Elf1 has also suggested that elongation factors may directly assist in the stabilization of Pol II intermediates through contacts with the histones. Spt4/5 and Elf1 cooperatively promote Pol II passage both by affecting the orientation of the Pol II clamp on the nucleosome and by direct contacts with the histone proteins.[67,75] Direct histone contacts may be a widespread mechanism of elongation factors on the nucleosome. Indeed, contacts between Paf1 and the H2A/H2B acidic patch have been detected and the elongation factor Spt6 has been shown to have histone chaperone activity.[76,77] Interestingly, despite the higher fraction of complexes stalled at SHL-1, no octamer transfer intermediates were detected in their CryoEM reconstructions.[67] It is possible that Spt4/5 and Elf1 cooperate to bias elongation through a pathway that does not involve octamer transfer (Figure 2(C)).

In addition to specific elongation factors, general histone chaperones also play a key role in transcription, and particularly the H2A/H2B chaperone FACT (FAcilitates Chromatin Transcription). FACT improves elongation in vitro and its depletion in vivo produces major elongation defects by all three polymerases.[53,78] Including FACT alone with Pol II transcription reactions promotes passage through the nucleosome and the formation of hexasomes.[78,79] However, dissociation of the dimer is not required for FACT function as neither covalent crosslinking of the histone octamer, nor destabilization of the histone dimer-tetramer interface inhibits FACT-dependent stimulation.[79] This has led to a model that FACT assists with Pol II passage mainly by facilitating disruption of nucleosomal DNA. Consistent with this, cryoEM structures combined with Hydrogen-Deuterium exchange experiments suggest that FACT binds to the DNA-binding surface of H2A-H2B, promoting DNA unpeeling.[80] A recent cryoEM structure of FACT bound to a nucleosome with Pol II arrested at SHL-4 shows FACT bound at SHL +1 instead of at the dyad.[75] As a result, it is possible that FACT interactions with the nucleosome may be highly plastic and adapt in response to Pol II passage. Interestingly, a kinetic analysis of FACT-assisted elongation has suggested that FACT reduces barriers throughout the nucleosome, but particularly between SHL −5 and SHL +2.[79] FACT has also been implicated in the reassembly of nucleosome structure in the wake of Pol II passage.[81] This reassembly function may be linked to the action of other factors (such as remodelers with nucleosome assembly activity) or elongation-associated histone modifications (such as H2B ubiquitination).[82,83] On a sub-nucleosome, FACT adopts multiple conformations linked to the presence or absence of a second H2A-H2B dimer.[80] This may facilitate the displacement or retention of the dimer during Pol II progression. With Pol II, FACT makes contact with the entry-side dimer, which may further stabilize its retention during Pol II passage.[75]

Local changes in DNA topology mediated by RNA polymerase play an important role in influencing transcription through the nucleosome. DNA nicks within the nucleosome, which relax torsional stress, can both promote and inhibit transcription through the nucleosome depending on their location.[84] With prokaryotic polymerase, nicks between the entry side and SHL-3 inhibit passage, while nicks between SHL −3 and SHL +3 facilitate passage. Nicks near the entry side of the nucleosome likewise inhibit Pol II nucleosome passage.[84] This supports a role for the buildup of local torsional stress in regulating Pol II passage through the nucleosome. Higher resolution Pol II-nucleosome structures are needed to visualize whether this stress results in changes in DNA topology. Also,

while it is clear that the buildup of torsional stress is critical for elongation, it is unclear how further accumulation of stress at different locations influences progression through the nucleosome. Further reconstitution studies of polymerase progression should be done on topologically constrained nucleosomal templates to better understand how DNA torsion and topology alters transcription on chromatin.

Several other properties of the polymerase, and the nascent RNA may play a key role in regulating transcription through the nucleosome. Notably phosphorylation of the CTD does not appear to directly impact chromatin transcription.[85] However, association of factors with the CTD, which can be linked to its phosphorylation state, is likely to allosterically influence core polymerase properties (as has been proposed to occur during termination of transcription).[86,87] Adjacent polymerases may also influence nucleosome remodeling properties. Transcription with tandem polymerases improves overall transcription efficiency through the nucleosome.[62,88] This is in part due to the remodeling activity of the leading polymerase but also due to prevention of backtracking of the leading polymerase by the trailing polymerase. Furthermore, multiple polymerases may cooperate through the accumulation of torsional stress, which can both destabilize nucleosome structure and directly improve polymerase elongation.[19,89] Finally, nascent RNA can impact transcription through the nucleosome through at least two types of mechanisms. Nascent RNA secondary structure can both positively and negatively influence polymerase pausing and backtracking[35,48] and R-loops may contribute to nucleosome remodeling as they are expected to generally destabilize nucleosome structure.[90,91]

ATP-dependent chromatin remodelers

Diverse activities mediated by diverse motors

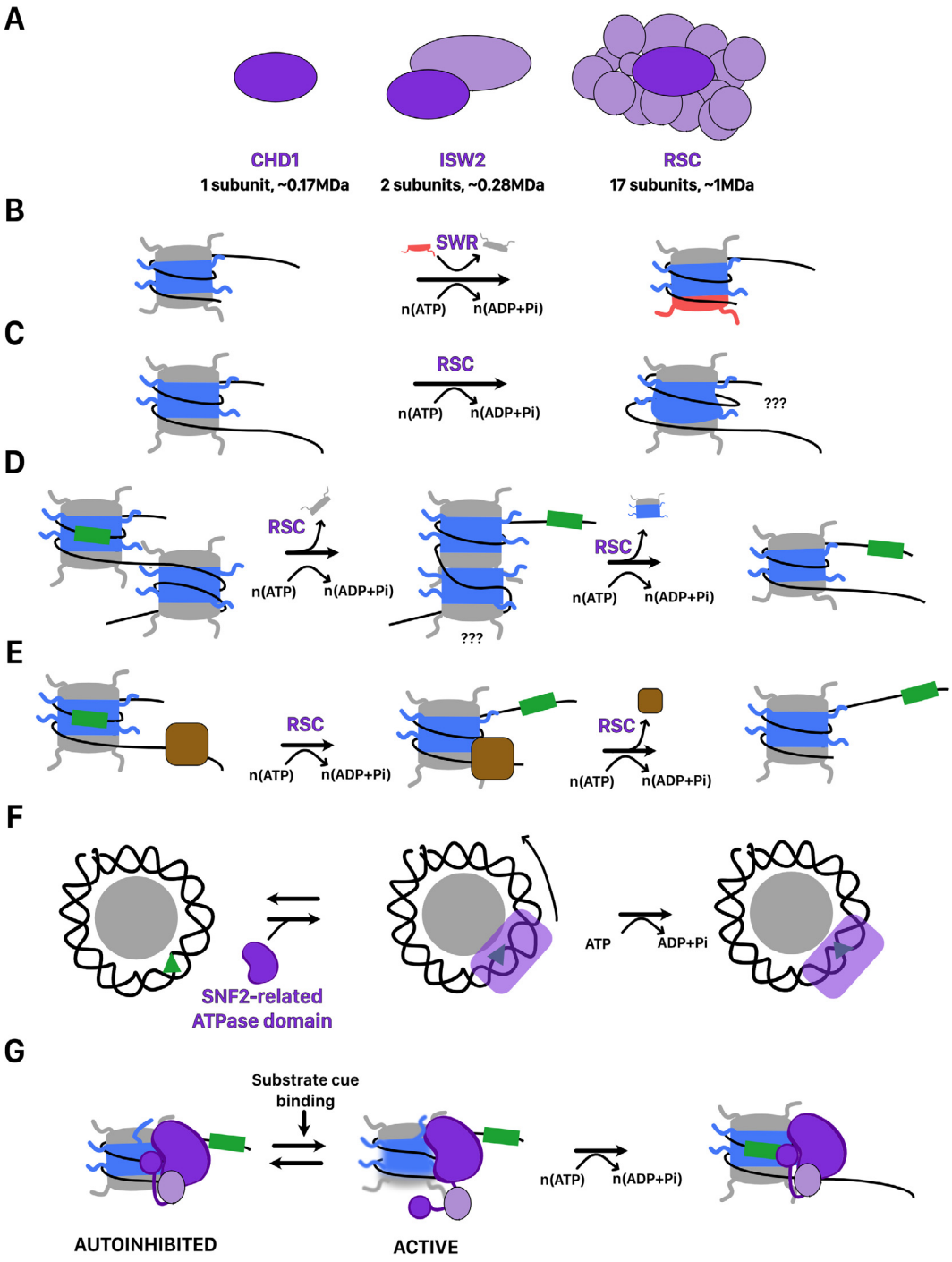
Although possessing considerable remodeling activity on its own, RNA polymerase is fundamentally limited in its remodeling capabilities. On the mechanistic side, this may reflect fundamental mechanical limitations imposed by the polymerase machinery. However, on the biological side, by being dependent on other factors RNA polymerases open themselves up for many regulatory possibilities. ATP-dependent chromatin remodelers are among the many factors that regulate RNA polymerase action on chromatin. Remodelers range in size from small single subunit motors to large multi-subunit complexes (Figure 3(A)).[6] They are able to catalyze a wide range of activities including most spontaneous transitions seen with the nucleosome alone (Figure 1(C)). However, remodelers do not necessarily act as “heat”, simply lowering the activation barrier to thermally inaccessible states.[7] This is because the irreversibility of ATP-hydrolysis allows structural changes produced by remodelers to be directional. For example, the SWR complex catalyzes the exchange of canonical H2A/H2B dimers with H2Az/H2B dimers but does not effectively catalyze the opposite reaction (Figure 3(B)).[92] Remodelers are also capable of creating stable non-canonical nucleosome structures not observed spontaneously. SWI/SNF-family remodelers catalyze the formation of stable structures called remosomes which contain an intact histone octamer but wrap ~30 bp more DNA than canonical nucleosomes and with overall weaker histone-DNA contacts (Figure 3(C)).[93,94] Some remodelers can also slide nucleosomes in order to displace DNA-bound proteins or other nucleosomes (Figure 3 (D)/(E)).[95,96] The wider range of transformations produced by remodelers when compared to RNA polymerase is likely linked to their fundamental activity of DNA translocation, which, as we discuss below, is a highly flexible means of disrupting nucleosome structure.

Structures and mechanisms of ATP-dependent chromatin remodeling enzymes

Common to all remodelers is the presence of a single subunit containing an ATP-hydrolyzing domain with homology to the yeast protein SNF2 from the SWI/SNF complex. Remodelers are further divided into families based on differences within this subunit.[97] These differences appear to determine the range of reactions catalyzed by the ATPase complexes. For example, complexes containing ISWI-family ATPases appear to only catalyze nucleosome sliding and nucleosome assembly, while SWI/SNF-family ATPase complexes catalyze a diverse range of reactions.[7] The ATPase domain of remodelers has two RecA-like lobes, which contact the phosphodiester backbone of DNA. ATP binding and hydrolysis occurs at the interface between the two lobes. Changes in the relative conformations of the RecA-like lobes driven by the ATPase cycle is proposed to power DNA translocation.

Biochemical and structural studies are highlighting both the commonalities and differences amongst remodelers in how they engage and transform nucleosomes. The ATPase domain of most remodelers associates with nucleosomal DNA at SHL ±2 (Figure 3(F)), with the only exception so far being the INO80 complex, which associates at SHL ±6.[7,98] Interestingly, structures of a truncated SNF2 ATPase subunit from the SWI/SNF complex suggest that this protein can associate with the nucleosome at both SHL ±2 and SHL ±6 suggesting that additional interactions outside of the ATPase domain may determine where the ATPase binds to the nucleosome.[99] From either of these locations, DNA translocation along the phosphodiester backbone appears to power the breaking of histone-

DNA contacts required for nucleosome remodeling. This is supported by the fact that single-stranded DNA gaps at these locations, which prevent translocation along DNA also prevent remodeling.[98,100–102] Binding of the SWI2/SNF2 ATPase to the phosphodiester backbone of nucleosomal DNA at SHL ±2 appears to amplify local deformations in the conformation of the DNA double helix caused by nucleosome formation (Figure 3(F)).[103] Such effects may exert sufficient torsional stress to locally destabilize histone-DNA contacts. Indeed, on naked DNA several remodelers have been shown to generate negative superhelical torsion.[104] However, it is important to note that remodeling of nucleosomes assembled on non-specifically nicked DNA, which globally relaxes superhelical torsion, is not inhibited.[105,106] This indicates that large-scale generation of superhelical torsion throughout the



nucleosome is not necessarily a core requirement for remodeling. Beyond the scale of monucleosomes, it has been shown that human and yeast SWI/SNF complexes can promote supercoiling changes in closed circular nucleosomal arrays.[107–109] Such changes can in principle be coupled with the torsional changes induced by RNA polymerases.

Despite the core similarities between remodelers, how translocation by the ATPase domains of remodelers is harnessed to create specific remodeling outcomes is likely to differ substantially and many of the details remain unclear. Nucleosome sliding has been proposed to occur by the propagation of helical twist defects around the nucleosome in a manner similar to what has been proposed for uncatalyzed nucleosome sliding (Figure 3(F)).[29,110] The resetting of histone-DNA contacts around the nucleosome may only require single translocation events. Indeed, fundamental ~1–2 bp increments of translocation can be observed during nucleosome sliding catalyzed by multiple remodelers.[111,112] Larger-scale disruptions of nucleosome structure such as nucleosome disassembly or histone exchange, which by necessity involve more extensive breaking of histone-DNA contacts, are likely to require more units of translocation and additional remodeler-nucleosome contacts to stabilize intermediates. For example, histone exchange by the SWR complex relies on contacts between its noncatalytic subunit Swc6 and flanking DNA to enable the loosening of contacts between the exiting H2A-H2B dimer and nucleosomal DNA.[113]

Remodelers also rely on dynamics in specific regions of the histone octamer core. The requirement for dynamics has been inferred from the combination of structural studies using techniques such as NMR and cryo-EM and from functional studies where restraining histone dynamics using site-specific disulfide crosslinking inhibits remodeling activity.[114–116] However, the histone dynamics required for remodeling appear to vary considerably between different remodelers. For example, H3-H4 crosslinking inhibits remodeling by the ACF complex but does not affect remodeling by the INO80 complex.[114] The use of octamer dynamics by remodelers may be a major distinguishing feature of their remodeling mechanisms when compared to RNA polymerases. So far, restraining histone octamer dynamics by crosslinking only appears to modestly, if at all, affect polymerase passage through the nucleosome.[53,79]

Outside of the core SNF2-related catalytic subunit, remodelers are frequently associated with several accessory proteins, which differ substantially between families (Figure 3(A)). These proteins often play integral roles in promoting or regulating the core activity of remodelers.[117] In the case of the SWI/SNF family BAF complex, several alternative complexes exist with different compositions each with different roles in promoting tissue specific gene expression.[118] Some subunits may also have additional catalytic activities including histone modifying or ATPase activity. For example, in addition to a remodeling ATPase, the NURD complex contains a histone deacetylase subunit and members of the INO80 family of remodelers contain the hetero-hexameric AAA + ATPase Rvb1/2.[119,120] While the histone deacetylase subunit of NURD is thought to act in concert with the remodeling ATPase to deacetylate nucleosomes, the Rvb1/Rvb2 ATPase in the INO80 complexes is thought to be involved in

Figure 3. Mechanisms of nucleosome remodeling by ATP-dependent chromatin remodelers. (A) Cartoon representations of selected remodelers from *S. cerevisiae.* In dark purple is the ATP hydrolysing subunit. Remodelers range in size from the single subunit remodeler CHD1 to the mega dalton sized 17-subunit RSC complex. (B) Directional histone exchange catalyzed by the SWR complex. H2A-H2B is exchanged for H2Az-H2B but not the reverse reaction. (C) Remosome formation catalyzed by the RSC complex. The structure of remosomes is unclear but involves ~30 bp of additional DNA wrapped around the core and looser histone-DNA contacts throughout the nucleosome. (D) Nucleosome disassembly catalyzed by the RSC complex. RSC sliding of a nucleosome into an adjacent nucleosome causes its disassembly. This may involve an overlapping dinucleosome intermediate recently described. The green box is used as a reference location on the DNA. (E) Transcription factor disruption by the RSC complex. RSC sliding of a nucleosome through a DNA-bound transcription factor causes its displacement. (F) DNA translocation by SWI2/SNF2 family chromatin remodelers binding at SHL 2. The green triangle is used as a reference location on DNA. Binding at SHL2 stabilizes twist defects at that location, allowing breaking of histone DNA contacts. Binding, hydrolysis, and/or release of a nucleotide allows the resetting of histone-DNA contacts. Further rounds of translocation with additional contacts is expected to enable more complex activities, such as histone exchange. (G) Regulation of remodeler activity by autoinhibitory domains. After binding the nucleosome, remodelers are held inactive by contacts with autoinhibitory domains on either the ATP hydrolyzing subunit or accessory subunits. Contacts with substrate cues relieve this autoinhibition and allow remodeling activity. The active state may be associated with increased conformational dynamics in the histone octamer. The green box is used as a reference point on the DNA.

complex assembly.[121] It is also possible that accessory subunits dynamically associate with the complex during a remodeling reaction. Some evidence for this possibility is suggested by ChIP-exo data, which has captured subcomplexes of INO80-family remodelers on chromatin.[122]

Principles of regulation

The process of remodeling can be divided into three stages in a manner analogous to transcriptional regulation. Like RNA polymerase, to initiate their activity remodelers must first be recruited to their relevant genomic locations at the proper time. After finding its target nucleosome, remodelers must commit to their remodeling activity. In the case of some activities, such as nucleosome sliding, this not only involves the decision to activate remodeling but also a commitment to a particular direction for the activity. Finally, after disrupting nucleosome structure, remodelers must have a means to sense the formation of an appropriate product and terminate further activity.

How remodelers achieve genomic specificity is unclear but is likely to involve the cooperative recognition of several chromatin features. In some cases, this may be achieved directly through subunits of the remodeling complex. Many remodelers contain histone reader domains in either the ATPase or accessory subunits which can recruit the remodeler to nucleosomes with a specific post translational modification state. For example, the SWR complex's accessory protein Bdf1 contains a bromodomain which recruits it to acetylated nucleosomes.[123,124] However, targeting of remodelers to specific loci may often depend on its interaction with other factors that provide specificity. Remodelers can directly associate with sequence-specific transcription factors or other reader-domain proteins (like HP1) to execute their function.[125,126] Recruitment can not only play an important role in selecting the appropriate nucleosome to disrupt, but also the directionality of activity. For example, transcription factor binding to the ISW2 complex helps set the direction of nucleosome sliding by this complex.[127]

After associating with their target nucleosomes, remodelers must dynamically integrate information about their substrate nucleosome and chromatin context to decide whether to proceed with remodeling. To accomplish this, a common theme across remodelers is regulation through autoinhibition. In the absence of their substrate, the ATPase subunit is held in an inactive conformation often through interactions with specific autoinhibitory domains (Figure 3(G)).[128–130] Binding to the appropriate nucleosome substrate can disrupt this inactive conformation. However, in some cases, even after binding the nucleosome autoinhibitory domains still prevent either ATP hydrolysis and/or the coupling of hydrolysis to remodeling.[131,132] Overcoming such autoinhibition may require a post-binding conformational change that enables contacts between the complex and specific substrate cues (Figure 3(G)). Common stimulatory substrate cues include accessible extranucleosomal DNA, the N-terminal tail of histone H4, and the H2A-H2B acidic patch. Sensing of these cues can be accomplished by direct interaction of the ATPase subunit with each cue, as appears to be the case for ISWI-family remodelers.[133] Alternatively, accessory subunits may also bind to these motifs and allosterically activate the ATPase domain. For example, the non-catalytic SMARCB1 subunit of BAF complexes contacts the acidic patch in order to activate remodeling.[134] Modification of substrate cues provides a simple but potent means to control remodeler activity both positively and negatively. For example, post translational modification of the acidic patch reduces remodeling by ISWI-family remodelers.[135]

Another interesting means of regulation is the association of multiple remodeling complexes to the same nucleosome. Because the nucleosome is two-fold pseudosymmetric, two complexes can in principle engage the same superhelical location on opposite faces of the nucleosome. For example, processive nucleosome sliding by the ACF complex is promoted by the cooperative association of two active complexes to a single nucleosome.[136] It has been proposed that a coordinated conformational switch prevents a futile tug of war between the two motors on the nucleosome.[137] Coordinating this switch may be achieved in part through asymmetric allosteric conformational changes in the octamer core.[138] This behavior may not be unique to ACF as the INO80 complex has also been reported to cooperatively associate with and remodel single nucleosomes.[139] It remains unclear what would result if two different remodeling complexes were to attempt to remodel the same nucleosome. Interestingly, the locations of several different remodeling families frequently overlap in ChIP-seq datasets, though further study will be needed to assess actual co-occupancy.[140]

The changes that specify termination of remodeling activity are very poorly understood. Loss of substrate cues directly associated with the remodeling reaction catalyzed might provide a means to directly communicate termination of remodeling. For example, H2Az-only containing nucleosomes do not stimulate ATP hydrolysis by SWR, likely because they lack a substrate cue unique to the L2 loop of canonical H2A which is recognized by the Swc5 subunit.[141] Alternatively, substrate cues may emerge after remodeling which inhibit further remodeling activity. Some ISWI-family remodelers have been demonstrated to bind adjacent nucleosomes in addition to its substrate nucleosome, which may control its activity.[142,143] Competition between remodelers and other proteins for binding substrate cues may also provide

an important means for terminating remodeling. For example, the H2A/H2B acidic patch, which is a common substrate cue for remodelers, is both bound by many non-remodeler proteins and by nucleosomes in trans via the H4 N terminal tail.[15]

Interface of Remodelers and RNA Polymerases at Different Stages of Transcription

Regulation of promoter chromatin architecture

Chromatin remodelers play an integral role in creation of promoter chromatin architecture by all three polymerases. This involves first the creation of a nucleosome depleted region (NDR) where sequence-specific and general transcription factors can assemble followed by the precise positioning of nucleosomes upstream and downstream of the transcription start site. Creation of an NDR appears to be an obligate feature of transcription initiation, since binding of TBP to DNA, which is required for all three polymerases to initiate, is greatly inhibited in the presence of nucleosomes.[144,145] However, chromatin architecture at the promoter plays additional key roles in coordinating events during initiation. Precise positioning of both the +1 and −1 nucleosome has been implicated in the cooperative assembly of the pre-initiation complex for Pol II and III-dependent genes.[146–149] This positioning of the +1 nucleosome may also be particularly important for transcription start site choice at some Pol II-dependent genes.[149]

Our best understanding of how nucleosome-positioning is achieved at promoters comes from several elegant studies of *S. cerevisiae* Pol II promoter architecture (Figure 4(A)). Using a library of genomic DNA, purified histones, remodelers, and/or transcription factors, nucleosome positioning at Pol II promoters has been faithfully reconstituted.[150] Careful analysis of positioning experiments with different combinations of remodelers has delineated at least two general pathways through which remodelers cooperate to create promoter architecture (Figure 4(A)). One pathway relies on the binding of sequence-specific transcription factors to create a barrier against which the +1 and −1 nucleosomes are positioned by the ISW2 complex. The second pathway relies on solely the ability of the INO80 complex to sense local DNA topology associated with promoter-proximal sequences to position the +1 and −1 nucleosomes.[150,151] Changes in DNA topology associated with RNA polymerase loading may cooperate with remodelers in further refining promoter chromatin architecture. Interestingly, neither histone modifications nor histone tails appear to substantially influence +1 positioning by INO80 as native, recombinant, and tailless histones are roughly equally sufficient for +1 positioning.[151] Nucleosomes downstream of the +1 nucleosome are then arranged in an evenly spaced nucleosome array, which is accomplished by remodelers with spacing activity such as ISW1b.[150]

The +1 nucleosome is also associated with an enrichment in the histone variant H2Az, particularly for poised or inducible genes.[36,152] This is thought to reduce the initial barrier to Pol II immediately after promoter escape. In cerevisiae, the SWR complex is recruited to the +1 nucleosome in a manner that depends heavily on its Swc2 subunit binding promoter-proximal DNA and to a lesser extent on Bdf1 binding histone acetylation (Figure 4(B)).[122,124,153] It is currently unclear whether these features are sufficient to directly recruit SWR to its substrate nucleosomes, or whether SWR depends on other factors for its recruitment. The opposite exchange reaction has been proposed to be catalyzed by INO80, but this activity remains debated.[98,154–156] Recent live-cell imaging experiments suggest instead that Pol II transcription directly displaces H2Az.[156] Interestingly, H2Az is enriched on the promoter distal side of the +1 nucleosome, which may facilitate its displacement by polymerase.[157]

After initiation, remodelers may also play a direct role in recycling transcription factors bound at the promoters. SWI/SNF-family remodelers have been shown to displace nucleosomes and transcription factors from DNA by sliding nucleosomes through them.[95,96] Based on the specific methods used, it has been proposed that in *S. cerevisiae* wide NDRs may be associated with with non-histone proteins or with a transient RSC-bound MNase-sensitive “fragile” nucleosome that can be used to displace nucleosomes and recycle transcription factors (Figure 4(C)).[158,159] Consistent with a role in transcription factor displacement, rapid depletion of a subunit of the RSC complex increases the dwell time of the Ace1p transcription factor on chromatin.[160]

Remodelers also play a key role in preventing transcription initiation by occluding NDRs. When recruited by specific transcription factors, the remodeler ISW2 in cerevisiae occludes NDRs by repositioning promoter nucleosomes.[161,162] In mammals, Pol I transcription is repressed by positioning of promoter nucleosomes over the NDR via the NoRC complex.[163] Additionally, remodelers play a role in preventing cryptic transcription. Deletion of several remodelers involved in even nucleosome spacing downstream of the TSS leads to the widespread upregulation of cryptic antisense transcripts.[164–166] Widespread nucleosome spacing over ORFs may be a general mechanism to repress cryptic promoters, which if allowed to proceed into active gene bodies can lead to transcription conflicts and inhibition of elongation.

Facilitated elongation through the gene body

Because of the intrinsic nucleosome remodeling activity of the transcriptional machinery, it is

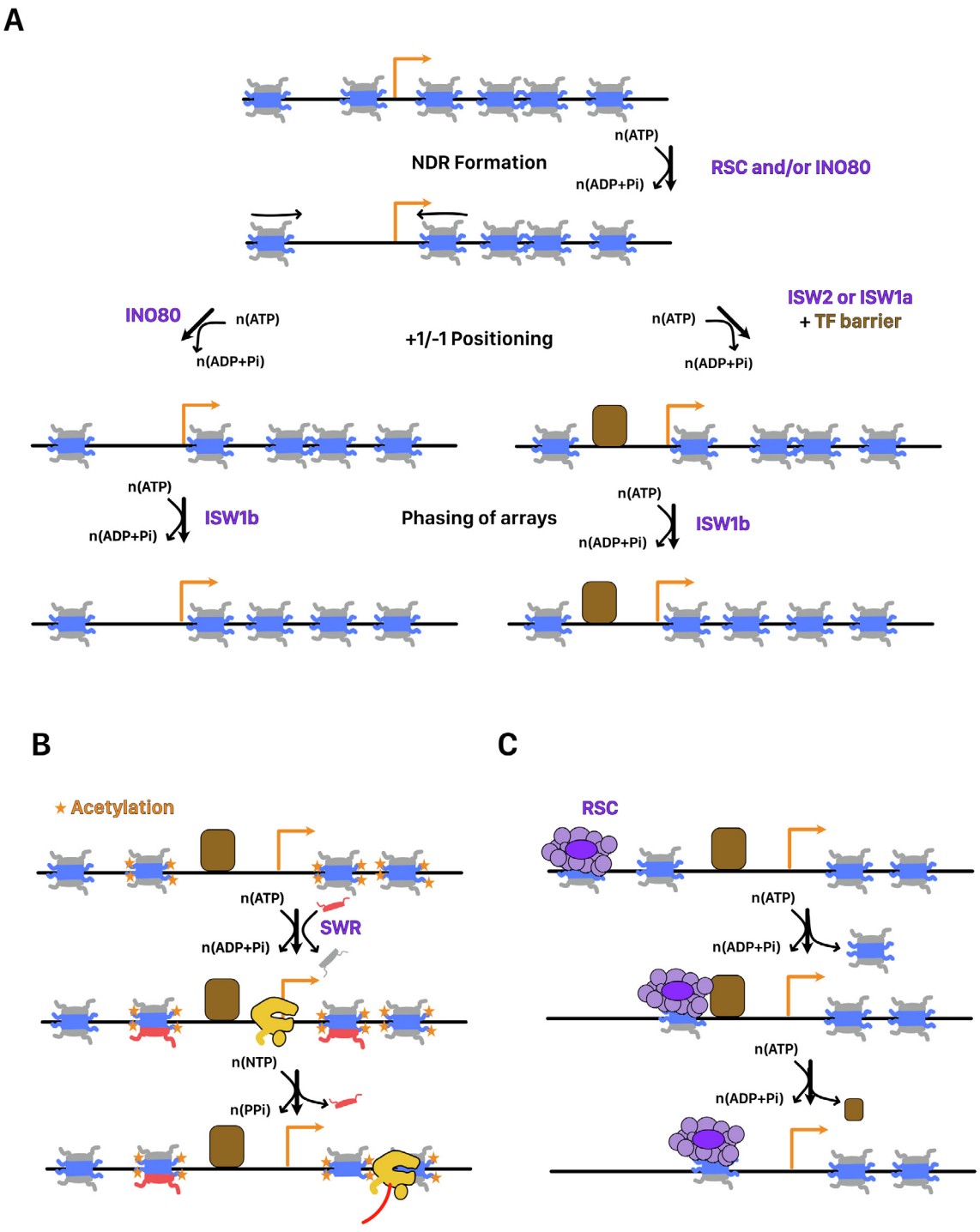


Figure 4. Chromatin remodeling near the transcription start site. (A) Two pathways for promoter chromatin architecture in *S. cerevisiae*. The NDR is first formed by the action of the RSC and/or INO80 complex recognizing sequence features near the promoter. The +1/−1 nucleosome is then positioned either by the INO80 complex using local DNA topology to position the nucleosome or by the ISW2 or ISW1a complexes positioning nucleosomes relative to a bound transcription factor. After establishment of the +1 nucleosome, ISW1b establishes a phased nucleosome array relative to the +1 nucleosome. (B) Establishment of H2Az nucleosomes at the +1/−1 nucleosomes. SWR recognizes histone acetylation and possible promoter DNA sequences to exchange H2A with H2Az dimers. H2Az has a marked preference for incorporation on the promoter distal side of the nucleosome. Transcription by Pol II may disassemble H2Az containing nucleosomes during passage. (C) RSC action at “fragile” nucleosome containing NDRs. RSC-bound “fragile” nucleosomes may be translocated to maintain a clear NDR or displace bound transcription factors.

possible that elongating RNA polymerase alone may be sufficient to transcribe through the gene body. Elongating polymerase is likely to be most self-sufficient at highly transcribed loci, where continued Pol II passage and accumulated supercoiling destabilizes nucleosome structure enough for elongation to proceed unimpeded.[19,50,62,88,89] However, at lowly expressed genes, or during a pioneering round of transcription, Pol II passage is likely to require assistance in moving through nucleosomes. Indeed, even in the presence of several elongation factors, reconstituted transcription reactions on nucleosomal templates under physiological conditions proceed at only a fraction of the 2 kb/min average elongation rate observed in vivo.[75,79,167] As a result, remodelers likely play a key role in both promoting the progression of polymerases through the gene body and maintaining chromatin structure after Pol II passage (Figure 5). Remodelers can, in principle, facilitate elongation by disassembling nucleosomes ahead of the polymerase completely or to hexasomes (Figure 5(A)). In vitro, inclusion of RSC and the H2A/H2B chaperone NAP1 in transcription reactions promotes the formation of a hexasome which improves transcription efficiency by Pol II.[168] Incorporation of histone variants that present a lower barrier to polymerase by remodelers, like H2Az or the mammalian-specific variant H2A. B, has also been suggested to improve elongation (Figure 4(B)).[169] Remodelers can also reposition nucleosomes off of sequences that present a high energetic barrier to nucleosomal passage (Figure 5 (B)). Repositioning of nucleosomes off of a high barrier sequence by ISW2 in vitro has been demonstrated to improve elongation efficiency.[170] Finally, remodelers may create stable conformational rearrangements in the nucleosome that facilitate Pol II passage (Figure 5(C)). The remosome produced by RSC could represent one such rearrangement.[93,94] Interestingly, RSC has recently been shown to not only be associated with promoter nucleosomes, but also with nucleosomes within the bodies of highly transcribed genes.[171]

Alternatively, remodelers may facilitate transcription elongation by directly acting on the elongating Pol II-nucleosome complex (Figure 5 (D)). CHD1 is a single-subunit chromatin remodeler that is required for efficient elongation.[75,172–174] CHD1 possess a DNA-Binding Domain (DBD) which engages extranucleosomal DNA and sets the directionality of nucleosome sliding as well as an ATPase domain that engages at SHL +2.[175] While nucleosome sliding could account for its role in elongation, we propose that CHD1 may also more directly assist in the disruption of nucleosome structure as Pol II transits the nucleosome.

Biochemical data suggests that CHD1 engages extranucleosomal DNA beyond SHL +7 to direct the sliding reaction.[175] However, Cryo-EM and biochemical data have revealed that CHD1 can also engage extranucleosomal DNA near SHL −7 with its ATPase domain still bound at SHL +2.[176–178] In this conformation, the DBD unpeels DNA at SHL-7 from the octamer surface.[177–179] This stable unpeeling may facilitate the entry of Pol II into the nucleosome. Pol II passage through the dyad requires disruption of DNA near SHL +2 which could be enabled by CHD1′s ATPase domain. As Pol II progresses through the nucleosome, it may displace the DBD from SHL-7, allowing interactions at SHL +7. Consistent with this, a structure with CHD1 bound to a Pol-II transcribed nucleosome arrested at SHL-4.5 suggest the DBD undocks from SHL-7 as Pol II enters the nucleosome.[75] Unlike many remodelers, CHD1 does not depend on the H2A/H2B acidic patch to activate remodeling, which could allow it to cooperate with Paf1 or FACT in facilitating Pol II passage.[180] Interestingly CHD1 can form a complex with both FACT and Paf1 and deletion of CHD1 suppresses FACT phenotypes.[172,173,181] How long CHD1 can remain associated with the nucleosome during Pol II passage is not known. Recent cryo-EM structures of Pol II-nucleosome complexes suggest that binding of CHD1 and FACT to a transcribed nucleosome is mutually exclusive.[75] However, additional conformational rearrangements in the remodeler not detected in the captured states and/or contacts with elongation factors could allow CHD1 to remain associated with the nucleosome during Pol II passage.

Finally, remodelers may also facilitate elongation by treating elongating polymerase as a substrate. The protein CSB (Cockayne Syndrome B or Rad26 in *S. cerevisiae*) possesses nucleosome sliding and disassembly activity but can also directly associate with RNA polymerase.[182,183] Recently it was shown that CSB stimulates transcription in an ATP-dependent manner that is separate from nucleosome sliding.[184] CSB accomplishes this by associating with both DNA at the transcription bubble and the DNA immediately upstream of the polymerase. This prevents polymerase backtracking and helps the polymerase ratchet through the nucleosome. CSB activity promotes progression of Pol II through the nucleosome with either nucleosome survival or complete disassembly (Figure 5 (E)). It is unclear whether this is a mechanism unique to CSB or whether other remodelers could also treat RNA polymerase as a substrate.

Transcriptional termination

Remodelers may also play important roles in transcription termination by Pols I and II.[185–187] One general theme is the creation of accessible sites for termination factors to assemble at the 3′ end of ORFs. Near Pol II termination sites at coding genes is a nucleosome depleted region surrounded by an evenly spaced array of nucleosomes.[147,188] In

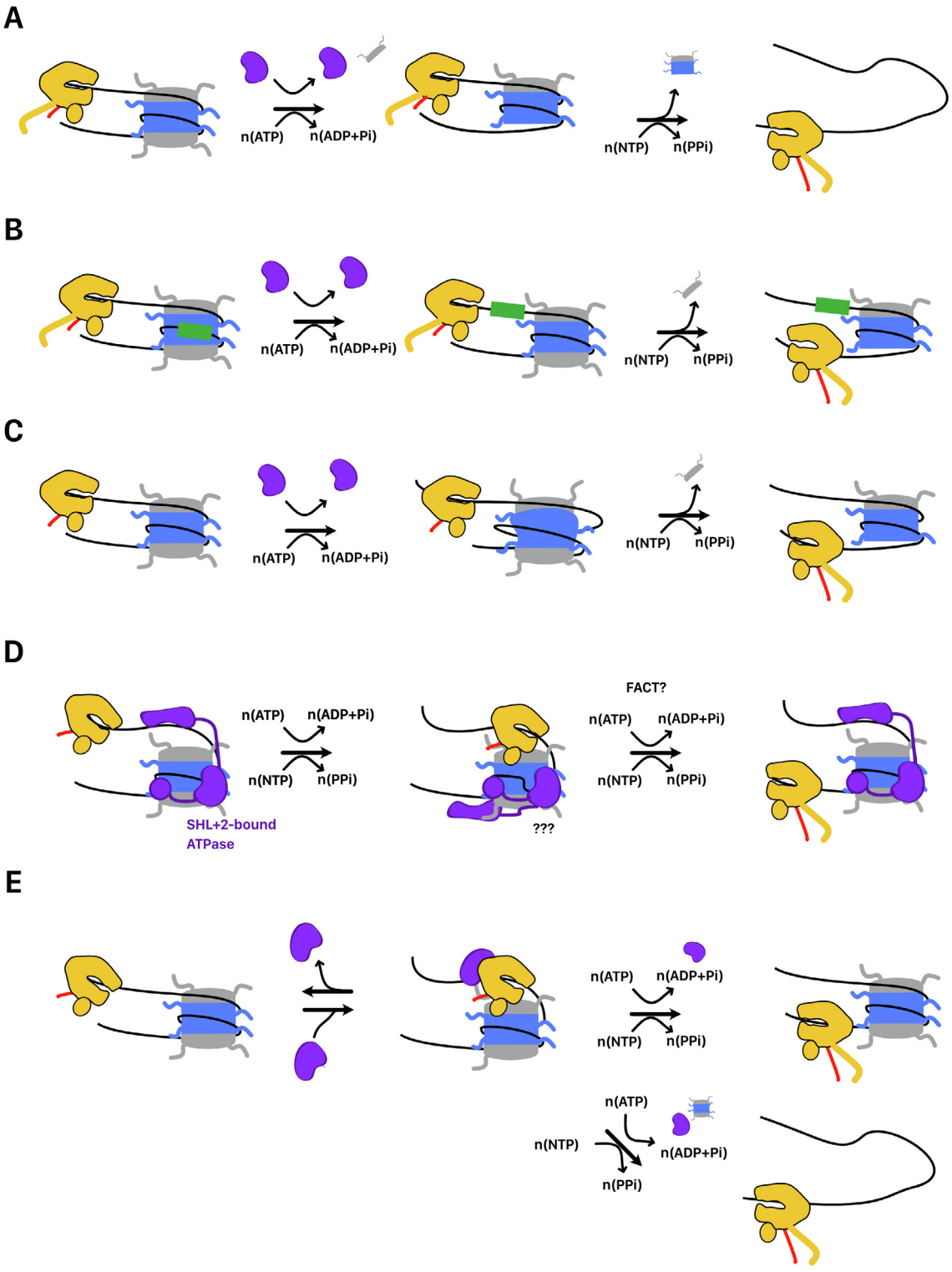


Figure 5. Chromatin remodeling during elongation. Remodelers can promote Pol II passage by (A) disassembling nucleosomes, (B) translocating nucleosomes off of high-barrier sequences, or (C) creating stable conformational rearrangements in nucleosome structure (e.g. remosomes). (D) Speculative model for promotion of elongation. Binding of extranucleosomal DNA by CHD1′s DNA binding domain stabilizes an unpeeled state that facilitates Pol II entry into the nucleosome. DNA translocation by the ATPase domain at SHL +2 may loosen histone DNA contacts needed to allow Pol II passage through the dyad. Contacts with FACT or other elongation factors (e.g. Paf1) may facilitate Pol II passage without CHD1 displacement. (E) ATP-dependent elongation activity of CSB (Rad26). CSB binds Nucleosome-associated Pol II. Translocation of the DNA upstream of Pol II by CSB prevents backtracking and promotes passage through the nucleosome resulting in either nucleosome disassembly or full nucleosome survival.

the *S. cerevisiae* genome, 3′ NDRs are found only weakly and are sensitive to the degree of MNase digestion. Additionally, *S. cerevisiae* 3′ NDRs correlate with closely spaced tandem genes and are not generally found at convergent genes.[159] This suggests that in *S. cerevisiae* the 3′NDRs may result simply from an extended 5′ NDR of a neighboring gene. However, in *D. melanogaster* S2 cells and human T cells, which both have substantially less densely packed genomes than *S cerevisiae*, the 3′ NDR is quite pronounced.[188,189] In humans the 3′ NDR also appears to correlate with polyadenylation site usage, which may allow the cleavage and polyadenylation factor (CPF) to access and process the nascent RNA during termination.[188] Formation of the 3′ NDR may be caused in part by DNA supercoiling due to elongating polymerase.[190] The 3′ NDR also possesses higher A/T content, which intrinsically disfavors nucleosomes.[159,188] However, in *S. cerevisiae* the NDR relies on the action of RSC, ISW1, and CHD1 and depletion of these remodelers can also cause termination defects.[187] How remodelers are recruited to these sites is unclear but direct interactions between remodelers and CPF, as has been reported for SWI/SNF complexes, could be responsible.[191]

Following termination sites, slowing elongating polymerase plays a key role in the process of termination as some mechanisms of Pol I and Pol II disassembly rely on kinetic competition between factors traveling along the nascent RNA transcript and further elongation.[87] For Pol I and Pol II coding transcription, termination can be achieved by the 5′-3′ exonuclease Rat1 which degrades the elongating transcript and, upon reaching the polymerase, displaces it from DNA. In contrast, Pol II noncoding transcripts are released by the RNA helicase Sen1, which translocates along the nascent transcript until it reaches and dissociates the elongating polymerase. Nucleosomes positioned by remodelers near the 3′ NDR may function as a roadblock to slow elongating polymerase and facilitate termination. Mutating histone-DNA contacts at the entry/exit sites of nucleosomes in *S. cerevisiae* results in widespread transcriptional termination defects and changes in pol II occupancy consistent with an increased elongation rate.[192]

In addition to these regulated termination pathways, there also exists pathways for clearing polymerase when it is terminally stalled by various roadblocks within the gene body. This is particularly relevant when the polymerase encounters DNA lesions that cannot be traversed. In this case, the stalled polymerase is polyubiquitinated, displaced, and degraded by the proteasome.[193] INO80 has been shown to associate with the AAA + ATPase Cdc48 and assist in the displacement of stalled Pol II in a manner that depends on INO80′s ATPase activity and ubiquitination of Pol II.[194] However, it is unclear whether INO80 assists in displacement through its nucleosome remodeling activity or through an undiscovered mechanism.

Outlook

Our understanding of the mechanisms of chromatin remodeling by both RNA polymerases and specialized ATP-dependent remodelers has advanced considerably in recent years. While new insights will continue to emerge from studying both of these motors' actions on chromatin in isolation, a major gap is how much the mechanism of remodeling by these two classes of motors is altered when both are associated with the same nucleosome. How and under what conditions do remodelers and polymerase directly synergize to disrupt chromatin structure? When their activities conflict, whose activity prevails and what determines who results as the "winner?" The continuing application of single molecule methodologies could be very useful as a means to assess what happens when these motors meet on a single nucleosome. Understanding the role of R-loops in regulating chromatin structure and remodelers is also a rich area of investigation. Interestingly, it was recently suggested that INO80 associates with and promotes the resolution of R-loops in cancer cells.[195]

Chromatin-based regulation of transcription also occurs on larger scales beyond that of the single nucleosome. Although chromatin was initially proposed to form a regular higher-order structure, multiple lines of evidence have converged on a view that chromatin structure is far more heterogeneous than initially anticipated.[196] In particular, the finding that nucleosome arrays can form liquid-like condensates underscores the complex and dynamic nature of higher-levels of chromatin organization.[197] While we are beginning to appreciate the myriad ways such condensates can influence genome compartmentalization, the environments within condensates may also dramatically influence both the properties and mechanisms of nuclear motors.[198] Further clarifying the range of packaging states adopted by chromatin and understanding which of their physico-chemical properties are compatible with transcription and chromatin remodeling are essential areas of investigation. Interestingly, the RNA polymerase CTD has been demonstrated to form liquid-like condensates, which may regulate Pol II's localization or activity.[199] Integrating insights from these disparate approaches holds immense promise toward our comprehensive understanding of the relationship between genome structure and transcription.

CRediT authorship contribution statement

Nathan Gamarra: Writing - original draft, Writing - review & editing, Conceptualization, Investigation.

Geeta J. Narlikar: Writing - review & editing, Supervision, Funding acquisition.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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